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A Taxonomic Study of the Fern Genus *Sphenomeris* (Lindsaeaceae) in Japan

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In order to revise the genus *Sphenomeris* in Japan, morphological, ecological, and cytological characters were examined. Japanese authors recognize four species, *S. biflora, S. chinensis, S. gracilis,* and *S. minutula* in Japan, whereas Kramer recognizes the former two species, reducing the latter two to *S. chinensis*. The present results show that each of *S. biflora, S. gracilis,* and *S. minutula* differs from *S. chinensis* in the habitat, basic chromosome number, rhizome habit, scales, texture and shape of leaves, glandular hairs, indusia, and/or spore wall ornamentation. Infraspecific cytotypes of *S. chinensis* are distinguished by the sizes of guard cells, spores, and glandular hairs. Our cytological and sporogenetic data, that putative hybrids between *S. biflora* and *S. chinensis* and between *S. chinensis* and *S. gracilis* have abnormal meiosis and irregular sporogenesis and are probably sterile, suggest that these three species are reproductively isolated, supporting the above species treatment. We describe a new species, *S. intermedia,* endemic to the southern Ryukyu Islands, which is suggested to be an allotetraploid species of hybrid origin between *S. biflora* and *S. chinensis* (2x). This species grows inland some distance from the coast, where the halophytic parent *S. biflora* grows.

Of 11 or probably more species of *Sphenomeris*, four species, *S. chinensis* (L.) Maxon, *S. biflora* (Kauf.) Tagawa, *S. gracilis* (Tagawa) Kurata, and *S. minutula* Kurata have been recognized in Japan by some authors. *Sphenomeris chinensis* is common along roadside or often at excavations, or sometimes in half-shaded forests, while *S. biflora* is a halophyte growing usually on coastal cliffs. These two species are widely distributed in the tropics and subtropics of the Old World, and in Japan they are distributed in southern central Honshu, Shikoku, Kyushu, the Bonin Islands, and the Ryukyu Islands. Morphologically intermediate, putative hybrids between the two species are rare where both parents occur adjacently (Nakaike 1982; Iwatsuki 1992). *Sphenomeris chinensis* is morphologically polymorphic with three varieties (Kramer 1967, 1971, 1972), cytologically variable and includes diploid and tetraploid aneuploids (Lin et al. 1990; references cited herein). *Sphenomeris chinensis* var. *kenzoana* (H. Ito) Nakaike was described from Shizuoka Prefecture, central Honshu, but it is probably extinct (Shimura pers. com.).

The other two species are endemic to Japan and restricted to very limited areas. *Sphenomeris gracilis* is a rheophyte known from Iriomote Island and Ishigaki Island, the Ryukyus (Tagawa 1937; Nakaike 1982; Iwatsuki 1992). *Sphenomeris minutula*, described from Amami-oshima Island, the Ryukyus, is an endang-

ered species confined to the Sumiyo river (Nakaike 1982; Iwatsuki 1992). Kramer (1972) doubted treatment of *S. gracilis* and *S. minutula* as separate species, because both are somewhat similar to small forms of *S. chinensis*. He tentatively considered *S. gracilis* to be conspecific with *S. chinensis*, and *S. minutula* as a permanently juvenile form of it. However, a recent study has shown a cytological difference between the species: *S. gracilis* and *S. minutula* have the basic chromosome number x=49, while Japanese *S. chinensis* has x=48 (Lin et al. 1990).

The objective of this study is to determine whether the four Japanese species of *Sphenomeris* can be recognized as separate species and whether *S. chinensis* is a single species or species complex. For this purpose, morphological and cytological examinations were performed for the four species and the putative hybrids between *S. chinensis* and *S. gracilis* and between *S. chinensis* and *S. biflora*. Sterility of hybrids was evaluated to determine the taxonomic status of the species.

Materials and Methods

Field surveys were conducted for ecological comparison and material collection during 1988 and 1993 through Japan, particularly in the Ryukyu Islands. All living materials used in the present cytological observations were collected from various localities of Japan (Table 1) and transplanted for cultivation in the Botanical Gardens, Faculty of Science, University of Tokyo. For the sake of comparison, four samples of *S. chinensis* were collected from Hainan, southern China. Herbarium specimens of *Sphenomeris* housed at the Botanical Gardens, Faculty of Science, University of Tokyo (TI), the Department of Forestry, Faculty of Agriculture, University of Tokyo (TOFO), and the Department of Botany, Faculty of Science, Kyoto University (KYO) were used for morphological observations (Appendix).

For observing the size and shape of stomata and glandular hairs, leaf blades were cleared with 1% NaOH for the plants that were cytologically checked by Lin et al. (1990). For examining spore ornamentation and size, untreated dried spores were collected from mature leaves, coated with gold, and observed using a JSM-8203S scanning electron microscope (SEM).

Several presumed hybrids were cytologically investigated following the method of Lin et al. (1990). Both gametic and somatic chromosome numbers and sporogenesis were examined for each hybrid. Somatic and gametic chromosomes were examined for additional materials of Japanese *Sphenomeris* which have not been examined before (Lin et al. 1990). For counting the chromosome number of *S. chinensis* from Hainan, fresh root-tips were fixed in the field in August, 1991. They were pretreated with 0.002 M 8-hydroxyquinoline solution under 20°C for 3–5 hours, and then kept in 70% alcohol at or below 10°C. The subsequent method employed followed Lin et al. (1990). They were fixed in acetic acid-alcohol (1:3) solution for 10 minutes, macerated in mixed solution of 1N HCl and 45% acetic acid (3:1) for 2 minutes at 60°C, stained by 2% aceto-orcein, and then squashed.

Results

Morphological and ecological comparisons The results of ecological observation and the comparison of morphological characters are shown in Table 2. Of the four species, *S. biflora* is distinct in the scales, leaf texture, leaf shape, indusia, and spore ornamentation as well as in the ecological features, as described by Kramer (1972) and others.

There are diploid and tetraploid cytotypes in *S. chinensis* (Lin et al. 1990). The diploids occur on excavations of roads in open places, as do tetraploids, or near the Urauchi river, Iriomote Island, the Ryukyus. The diploids are uniform in the following characters: the stipes red or dark brown, the leaves chartaceous, the scales 2–3-cell-rowed at base, the blade ovate-

Table 1. Materials of *Sphenomeris* examined and cytological data obtained

Taxon and locality	No. of plants	Chromosome no.	
		2n	n
S. biflora (2x)			
Haemi, Iriomote Is., Okinawa Pref.	3	96	48
Toyohara, Iriomote Is., Okinawa Pref.	1	96	48
Ohara, Iriomote Is., Okinawa Pref.	2	96	48
Shirahama, Iriomote Is., Okinawa Pref.	1	–	48
Sonai Peninsula, Iriomote Is., Okinawa Pref.	1	–	48
Tojo, Amami-oshima Is., Kagoshima Pref.	1	96	–
Suko, Amami-oshima Is., Kagoshima Pref.	2	96	–
Uken, Yamakawa-cho, Kagoshima Pref.	1	96	–
Mt. Asatachi, Chichijima Is., Bonin Isls.	1	96	–
Chichijima Is., Bonin Isls.	1	c.96	–
S. chinensis var. *chinensis* (2x)			
Shirahama, Iriomote Is., Okinawa Pref.	1	–	48
Mariudo, Urauchi River, Iriomote Is., Okinawa Pref.	2	–	48
Urauchi bridge, Iriomote Is., Okinawa Pref.	5	96	48
S. chinensis var. *chinensis* (4x)			
Komi, Iriomote Is., Okinawa Pref.	1	c.194	–
Hinai waterfall, Iriomote Is., Okinawa Pref.	1	–	>95
Hinai river, Iriomote Is., Okinawa Pref.	2	–	>95
Mt. Banna, Ishigaki Is., Okinawa Pref.	1	c.192	c.96
Mt. Omoto, Ishigaki Is., Okinawa Pref.	2	–	c.96
Tainoko river, Yakushima Is., Kagoshima Pref.	1	>190	–
Nakabashi, Yakushima Is., Kagoshima Pref.	1	>190	–
Mt. Gongen, Wushifuka, Kumamoto Pref.	2	>190	–
Mt. Takeyama, Yamakawa, Kagoshima Pref.	1	>190	–
Sakurajima, Kagoshima Pref.	1	>190	–
Chichijima Is., Bonin Isls.	1	>190	–
Oshima Is., Izu Isls.	1	–	>94
Yokosuka, Kanagawa Pref.	2	>190	–
Mt. Diaoluo, Hainan Is., China	2	c.188	–
S. chinensis var. *rheophila* (2x)			
Mt. Diaoluo, Hainan Is., China	2	94	–
S. gracilis (2x)			
Aira river, Iriomote Is., Okinawa Pref.	2	98	–
Maira river, Iriomote Is., Okinawa Pref.	3	98	–
S. intermedia (4x)			
Komi, Iriomote Is., Okinawa Pref.	1	–	c.96
Mt. Banna, Ishigaki Is., Okinawa Pref.	1	c.192	c.96
Mt. Omoto, Ishigaki Is., Okinawa Pref.	2	–	c.96
S. chinensis × *S. gracilis*			
Mouth of Hinai river, Iriomote Is., Okinawa Pref.	2	97	MI *
Kampira, Urauchi river, Iriomote Is., Okinawa Pref.	5	97	MI
S. chinensis (2x) × *S. biflora*			
Urauchi bridge, Iriomote Is., Okinawa Pref.	2	96	MI
Shirahama, Iriomote Is., Okinawa Pref.	1	96	MI
S. chinensis (4x) × *S. biflora*			
Hachijo Is., Izu Isls.	1	144	MI

*MI: meiosis irregular.

Table 2. Comparison of diagnostic characters of five Japanese *Sphenomeris* species and interspecific hybrids

Character	*S. biflora*	*S. chinensis*	*S. gracilis*	*S. minutula*	*S. intermedia*	*S. chinensis* (2x) × *S. gracilis*	*S. chinensis* (2x) × *S. biflora*	*S. chinensis* (4x) × *S. biflora*
Ecology	halophyte	various	rheophyte	rheophyte	open inland place	near river	open inland place	mountain path
Basic chromosome number	x=48 (2x)	x=48 (2x, 4x)	x=49 (2x)	x=49 (2x)	x=c.96 (4x)	2n=97 (2x)	2n=96 (2x)	2n=144 (3x)
Rhizome	short creeping	short creeping	creeping	creeping	short creeping	short creeping	short creeping	short creeping
Number of cell rows at scale base	4–6	2–3(–4)	2–3	2–3	2, 4(5)	2–3	2–5	3–5
Scale length (mm)	3.0–3.5	2–4	1.0–1.5	1	2–3	2–3	2–3	2–3
Scale shape	triangular-lanceolate	acicular-linear	narrow-triangular	narrow-triangular	acicular, triangular-lanceolate	acicular	acicular, triangular-lanceolate	acicular, triangular-lanceolate
Lamina texture	thick-coriaceous	chartaceous	thin-herbaceous	thin-herbaceous	subcoriaceous	thin-chartaceous	coriaceous	chartaceous
Length ratio of stipe to blade	1/3–1	1/4–1	1	1/3–1/4	≤1	1	1/3–1	1/4–1
Stipe color	brown	red(dark)-brown	stramineous	stramineous	reddish	pale brown	red	brown
Pinna shape	deltoid-lanceolate	lanceolate	(elongate) ovate	obliquely flabellate	oblong-lanceolate	lanceolate	ovate-lanceolate	ovate- and deltoid-lanceolate
Basal pinna	longest	shorter than upper one	shorter than upper one	shorter than upper one	shorter than upper one	shorter than upper one	various	various
Glandular hair	absent	present	present	present	rare/dense	present	rare	rare
Indusium	convex	convex or slightly convex	flat	flat	convex	flat	convex	convex
Spore wall ornamentation	semi-regulate	cerebroid-psilate	psilate	psilate	–	variable	variable	variable
Spore size (μm)	50 × 40	44 × 29(2x) 55 × 39 (4x)	45 × 27	45 × 33	–	variable	variable	variable

lanceolate, and the spore ornamentation cerebroid. However, it is difficult to discriminate the diploids and the tetraploids, using solely gross-morphological characters such as the size and shape of leaves, because the tetraploids show a wide range of variation in these characters, which always covers that of the diploids.

The sizes of guard cells, spores, and bicellular glandular hairs were found useful to distinguish the cytotypes (Table 3). Both guard cells and spores of the tetraploids are larger than those of the diploids, although their ranges overlap to some extent. The glandular hairs of the tetraploids are always longer than those of the diploids.

There is an intermediate form between *S. chinensis* and *S. biflora*, which shows combined or intermediate characters of the two species (Fig. 6). The form has three types of scales: acicular, linear scales with 2-cell-rowed base (*S. chinensis* type), triangular-lanceolate scales with 4(5)-cell-rowed one (*S. biflora* type); and acicular scales with triangular base 4(5)-cell-rowed one. In the form, the lamina is subcoriaceous, (ovate or) oblong-lanceolate; the basal pinnae shorter than the second basal; the pinnae bipinnate + pinnatisect; the bicellular glandular hairs about 160 μm long; guard cells 62–80 μm long, and indusia (1)2 cells thick, round-entire or dentate at distal edge. Thus, morphological comparison suggests that the intermediate form is perhaps of hybrid origin between *S. chinensis* and *S. biflora*. It is tetraploid, as described below.

Sphenomeris gracilis differs from *S. chinensis* in the rheophily and in the creeping rhizomes, narrowly triangular scales (1–1.5 mm long), stramineous slender stipes, and thin-herbaceous and elongate-ovate lamina (Table 2, Fig. 1). Some plants of *S. chinensis* growing together with *S. gracilis* in rheophytic habitat, are similar to *S. gracilis* in the thin-chartaceous, elongate-ovate, and small leaves (5–15 cm long), narrowly cuneate lobes sometimes with sinuate margin, but differ from it by the short-creeping rhizomes with clustered leaves, the reddish-brown or brown stipes, the acicular scales (2 mm long), and the lanceolate pinnae.

Sphenomeris minutula is the smallest-sized species growing on stream rocks only in the Sumiyo river. Plants cultivated in good condition have 3–10 times larger leaves than the wild plants (Fig. 1A). In the wild plants the leaves are 2–3 cm long, ovate or oblong, bipinnate to tripinnate, and have 2–4 pairs of

Table 3. Morphological comparison between two cytotypes of *S. chinensis*

Character	Diploid mean±SD (range)	No. of material (No. of plants)	Tetraploid mean±SD (range)	No. of material (No. of plants)
Guard cell length (μm)	54±8.4 (40–70)	90 (9)	65±5.3 (55–75)	90 (9)
Spore length (μm)	44±3.3 (37–53)	45 (5)	55±3.9 (45–65)	65 (7)
Spore width (μm)	29±2.7 (26–33)	45 (5)	39±3.8 (32–45)	65 (7)
Length of glandular hair (μm)	117±10.5 (107–127)	90 (9)	149±6.5 (133–160)	90 (9)

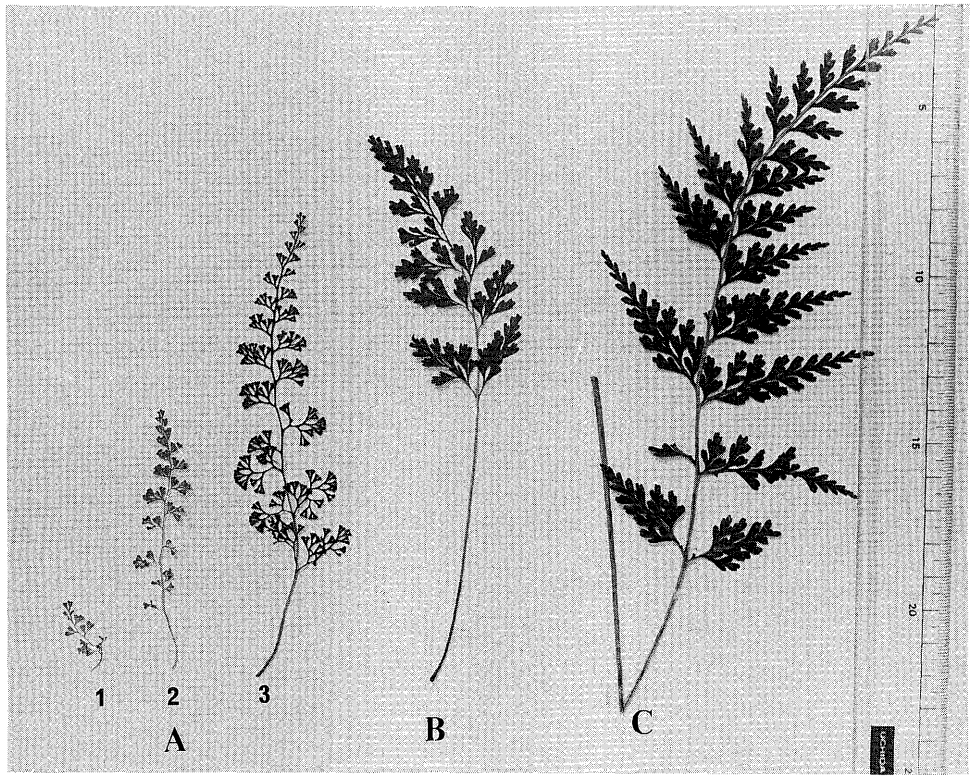

Fig. 1. Leaves of *S. minutula* (A), *S. gracilis* (B) and *S. chinensis* (2x) (C). A1, wild plant; A2, A3, cultivated plants.

pinnae (Fig. 1, A1), while in the cultivated plants the leaves are 3–14 cm long, narrowly deltoid-oblong or elongate-oblong, and have 4–10 pairs of pinnae (Fig. 1, A2, A3). In the fully fertile leaves in cultivation, the blade is tripinnate at base. One diagnostic character of *S. minutula* is the obliquely flabellate, deeply incised pinnae and narrowly cuneate lobes, which are stable irrespective of wild or cultivated condition (Fig. 1A). Another diagnostic character is spore shape and ornamentation (Fig. 2). The spores are ellipsoid, compared to the elongate bean-shaped spores of *S. gracilis*. The psilate spore ornamentation is very similar to that of *S. gracilis*, but differs from the semiregulate one of *S. biflora* and the cerebroid one of *S. chinensis* (2x). The spore size is similar to that of *S. gracilis* (Table 2).

Twenty-two plants of a putative hybrid between *S. chinensis* (2x) and *S. gracilis* were found to occur in streambed in the Urauchi river, Iriomote Island, the Ryukyus, where both *S. gracilis* and diploids of *S. chinensis* occur side by side. It is intermediate between the two species in that: the rhizomes are short-creeping with leaves somewhat clustered, the stipes pale-brown, and the pinna-lobes cuneate (Table 2). On the other hand, the hybrid is more similar to *S. gracilis* in having stipes slender and as long as or longer than the blade, and blade thin-herbaceous, elongate-ovate or ovate-lanceolate.

Twelve plants of a putative hybrid between *S. chinensis* (2x) and *S. biflora* were collected along a road on a slope near the Urauchi river, Iriomote Island, the Ryukyus. It is intermediate between *S. chinensis* and *S. biflora* in such characters as: the

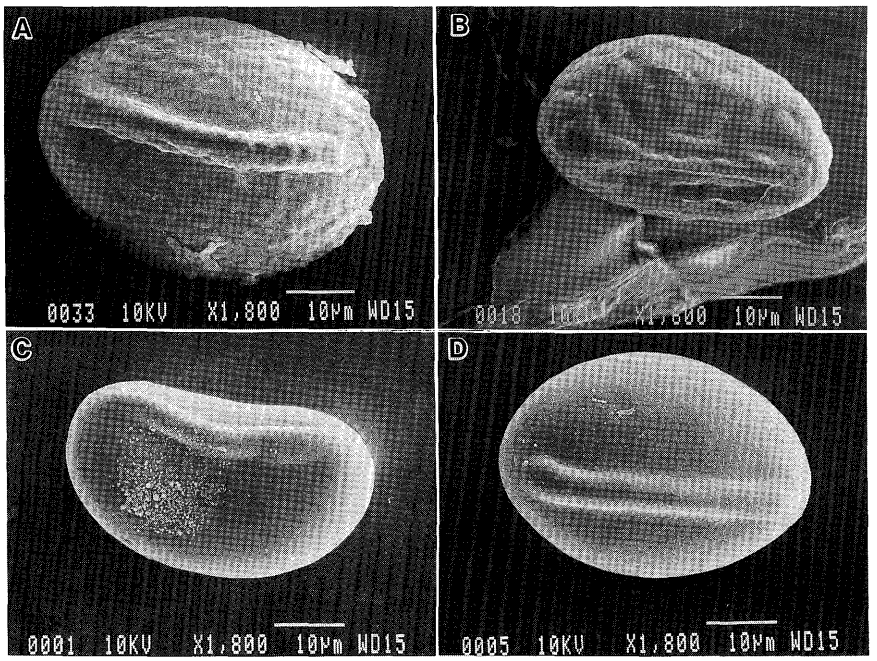

Fig. 2. SEM micrographs of spores. A. *S. biflora*. B. *S. chinensis* (2x). C. *S. gracilis*. D. *S. minutula*. Bars=10 μm.

scales acicular with triangular, 2–5-cell-rowed bases, the leaves 20–50 cm long, the stipes reddish, the lamina ovate-lanceolate and thick-coriaceous, the lowest pinnae shorter than the upper, the bicellular glandular hairs very rare on the lower surface of lamina, and the indusia thick and convex (Table 2).

One plant of a putative hybrid between *S. chinensis* (4x) and *S. biflora* grows by a path on Mt. Mihara, Hachijo Island, the Izu Islands, where tetraploids of *S. chinensis* and *S. biflora* occur. It is morphologically intermediate between the parents (Table 2). The scales are either 3-cell-rowed as in *S. chinensis* or 5(6)-cell-rowed as in *S. biflora*. The leaves are also dimorphic: three leaves are deltoid-ovate with the longest basal pinnae, as in *S. biflora*, while five other leaves are oblong-lanceolate with the basal pinnae shorter than the upper, as in *S. chinensis*. The bicellular glandular hairs are very rare or absent. The hybrid is triploid, as described below.

Cytology and sporogenesis Chromosomal data obtained are summarized in Table 1. The putative hybrid between *S. chinensis* and *S. gracilis* had 97 chromosomes in the somatic cells (Fig. 3A). The number is equal to the sum of the gametic chromosome numbers, n=48 and n=49, of its parents. Meiotic division was abnormal: 6–17 univalents appeared at metaphase I in the spore mother cells, and bridges and laggards were found in anaphase I (Fig. 3B). Each sporangium produced 32 spores typical of *Sphenomeris*, but the spores were irregular in shape (Fig. 3C). The hybrid seems to be sterile.

The putative hybrid between *S. chinensis* and *S. biflora* from Iriomote Island, the Ryukyus, had mitotic chromosomes of 2n=96, which is equal to the sum of the gametic chromosome numbers of diploids of *S. chinensis* (n=48) and *S. biflora* (n=48) (Fig. 3D). Meiosis was irregular and 4–6 chromosomes were univalents (Fig. 3E). Each sporangium produced 32

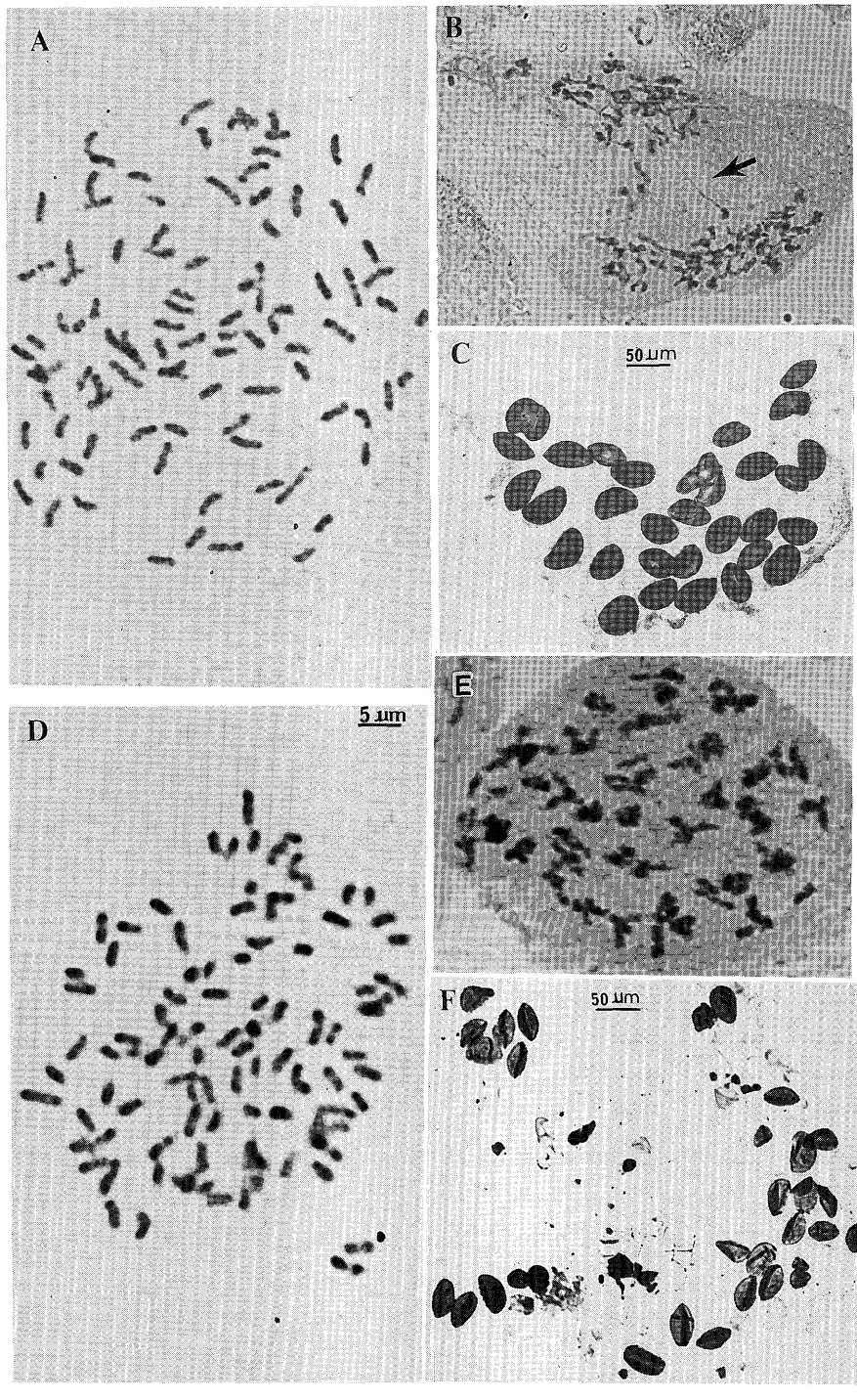

Fig. 3. Chromosomes and spores of putative hybrids. A–C. *S. chinensis* (2x) × *S. gracilis*. A. Somatic chromosomes of 2n=97. B. Irregular meiosis at telophase I showing chromosome bridge (arrow). C. 32 irregular spores produced in sporangium. D–F. *S. chinensis* (2x) × *S. biflora*. D. Somatic chromosomes of 2n=96. E. Bivalents and univalents in abnormal meiosis. F. 32 irregular spores produced in sporangium.

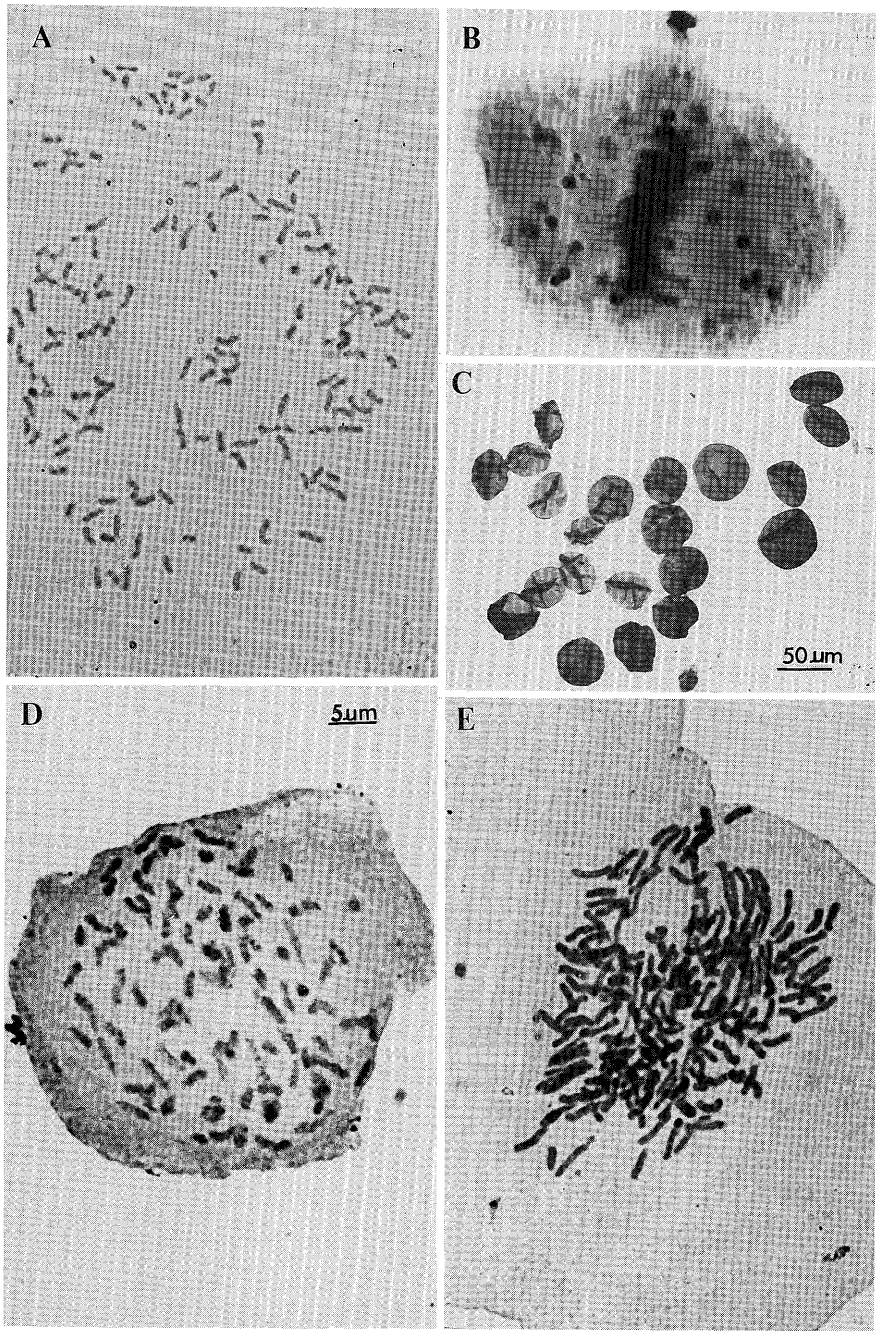

Fig. 4. Chromosomes and spores. A–C. Putative triploid hybrid *S. chinensis* (4x) × *S. biflora*. A. Somatic chromosomes of 2n=144. B. Abnormal meiosis showing many univalents. C. Irregular-shaped spores. D. Somatic chromosomes 2n=94 of *S. chinensis* var. *rheophila* from Hainan. E. Somatic chromosomes 2n=c.188 of *S. chinensis* var. *chinensis* from Hainan.

spores, which were irregular in size and shape (Fig. 3F).

The putative hybrid of *S. chinensis* (4x) and *S. biflora* collected from Hachijo Island, the Izu Islands, had 144 chromosomes in the somatic cells, which are euqal to the sum of the gametic chromosome numbers of tetraploids of *S. chinensis* (n=96) and *S. biflora* (n=48) (Fig. 4A). Meiotic division was abnormal and the spores were very irregular (Fig. 4B, C). It is probably sterile.

The intermediate form between *S. chinensis* and *S. biflora* noted above had somatic chromosomes of 2n=c.192 (Fig. 5) and gametic chromosomes of n=c.96, indicating that the form is tetraploid. Four to six univalents were observed in some meiotic divisions, and irregular spores were produced in about 10% sporangia.

The other cytological data obtained here (Table 1) verify the cytological feature of each species reported in an earlier paper (Lin et al. 1990).

Four individuals of *S. chinensis* collected outside Japan from Mt. Diaoluo, Hainan, southern China, were also cytologically examined. Two of the four plants grew on rocks in river bed and were identified as *S. chinensis* var. *rheophila* Kramer. It is a new record from China. They had somatic chromosomes of 2n=94 (Fig. 4D). The other two plants of var. *chinensis* growing in an evergreen broad-leaved forest had somatic chromosomes of 2n=ca.188 (Fig. 4E). The basic chromosome number, x=47, of the Hainanese plants differs from that of Japanese species (x=48 or x=49).

Discussion

Cytotypes of S. chinensis *Sphenomeris chinensis* shows a cytological variation as well as morphological and ecological ones. Wagner (1963) reported that a Hawaiian diploid has chromosomes of n=47, while Lin et al. (1990) showed that a Japanese diploid has chromosomes of n=48. Var. *rheophila* from Hainan examined here has chromosomes of 2n=94, the same number as that of the Hawaiian plants. Thus, there are different basic chromosome numbers in the diploids of *S. chinensis*. Since a diploid is also present in Ambon Island, the Moluccas (eastern Indonesia) (unpublished data), diploids of *S. chinensis* are perhaps more widely distributed than we are aware at present.

The tetraploids are more widely distributed than the diploids, although different chromosome numbers were reported from various areas; n=c.94, 96, c.98, c.100 and 2n=188, c.192, c.194, 196 (Manton and Sledge 1954; Mehra and Khana 1959; Roy and Pande 1962; Kurita and Nishida 1963; Bir 1965; Mitui 1968, 1973; Roy and Rao 1985; Lin et al. 1990; Kato et al. 1992). The tetraploids are very variable in the shape, texture and size of leaves, scales, indusia, and spores. Although Japanese diploids have uniform morphological characters, they fall within the range of those of the tetraploids. Differences between the diploids and tetraploids are found in the sizes of glandular hairs, spores, and guard cells. It is considered that cell size is correlated with ploidy level, as in other ferns (Barrington et al. 1986).

Taxonomy of Sphenomeris in Japan The chromosome numbers equal to the sum of those of the parents, the abnormal meiosis, and the abortive spores as well as the morphological intermediacy are useful to determine putative parents of the three hybrids. The first is a hybrid with 2n=97 between diploid *S. chinensis* (n=48) and *S. gracilis* (n=49); the second is one with 2n=96 between diploid *S. chinensis* (n=48) and *S. biflora* (n=48); and the third is one with n=144 between tetraploid *S. chinensis* (n=96) and *S. biflora* (n=48). All the hybrids are apparently sterile, suggesting that genetic isolation exists between *S. chinensis* and *S. gracilis* and between *S. chinensis* and *S. biflora*.

Probable hybrid sterility, as well as morphological and cytological differences, seems to indicate that *S. gracilis*, like *S. biflora*, is not conspecific with *S. chinensis*. *Sphenomeris gracilis* is similar ecologi-

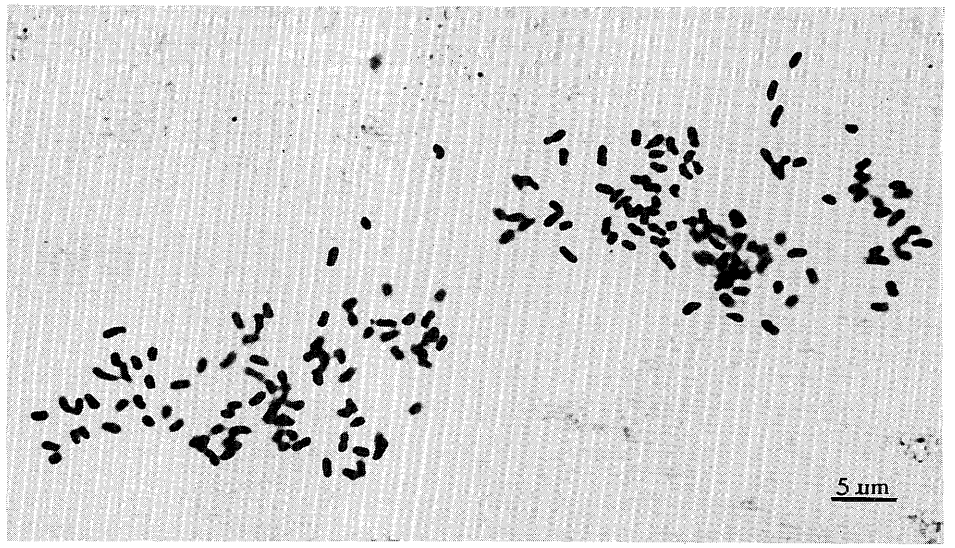

Fig. 5. Somatic chromosomes of *S. intermedia*, 2n=c.196.

cally and morphologically to *S. chinensis* var. *rheophila*. The small to medium-sized (10–20 cm long) leaves, slender stipes, thin, ovate-elongate or ovate blades, narrowly cuneate lobes, and bean-shaped spores are common to both species, and the characters excepting spore morphology are adapted to the rheophytic habitat. However, *S. chinensis* var. *rheophila* is distinguishable from *S. gracilis* by having short-creeping rhizomes, acuminate or long-acuminate scales, lanceolate pinnae, and chromosomes of n=47. Further study is required to investigate the relationship between the two with ecological similarity.

Sphenomeris minutula and *S. gracilis*, endemic to northern and southern islands of the Ryukyus, respectively, grow in somewhat similar habitats on mossy rocks in streambed. Kurata (1965) stated, describing *S. minutula* as a new species, that it is allied to *S. gracilis*. Although Kurata pointed out that *S. gracilis* differs from *S. minutula* in having more decompound and decidedly larger plants with about 10 pais of pinnae, *S. minutula* is capable to become as large as *S. gracilis* in good cultivated condition. The present comparison shows that *S. minutula* differs distinctly from either of *S. gracilis*, *S. chinensis*, and *S. biflora* in its obliquely flabellate pinnae, narrowly cuneate and deeply incised segments, and lamina 3–4 times as long as stipe in fully grown plants. Although *S. minutula* was tentatively regarded as a permanently juvenile form of *S. chinensis* by Kramer (1972), we consider that *S. minutula* is a species independent of *S. chinensis* and *S. gracilis*.

The tetraploid is suggested to be an allotetraploid from *S. chinensis* and *S. biflora*, because of its ploidy level and morphological intermediacy between the species. It is morphologically very similar to the hybrid between the two species. It occurs in Iriomote Island and Ishigaki Island, the southern Ryukyu Islands, where the putative parents are distributed. Since the tetraploid can be morphologically discriminated from the other species, we describe it as a new species *S. intermedia*.

Sphenomeris intermedia Lin, Kato et K. Iwatsuki, sp. nov. (Fig. 6).

Species inter *S. chinensis* et *S. bifloram* intermedia et vero similiter ex hybridatione harum specierum

ortae. Ab *S. chinensis* squamis triangularibus ad basin cellulis latis; lamina subcoriacea, nitida; indusiis bistrato-cellulosis differt. Ab *S. biflora* lamina oblongo-lanceolata; pinnis infimis brevior quam superioribus; stipitibus in vivo rubellis, in sicco atro-brunneis differt.

Rhizomes short-creeping. Scales 3–5 mm long, consisting of three forms, one form acicular, 2-cells

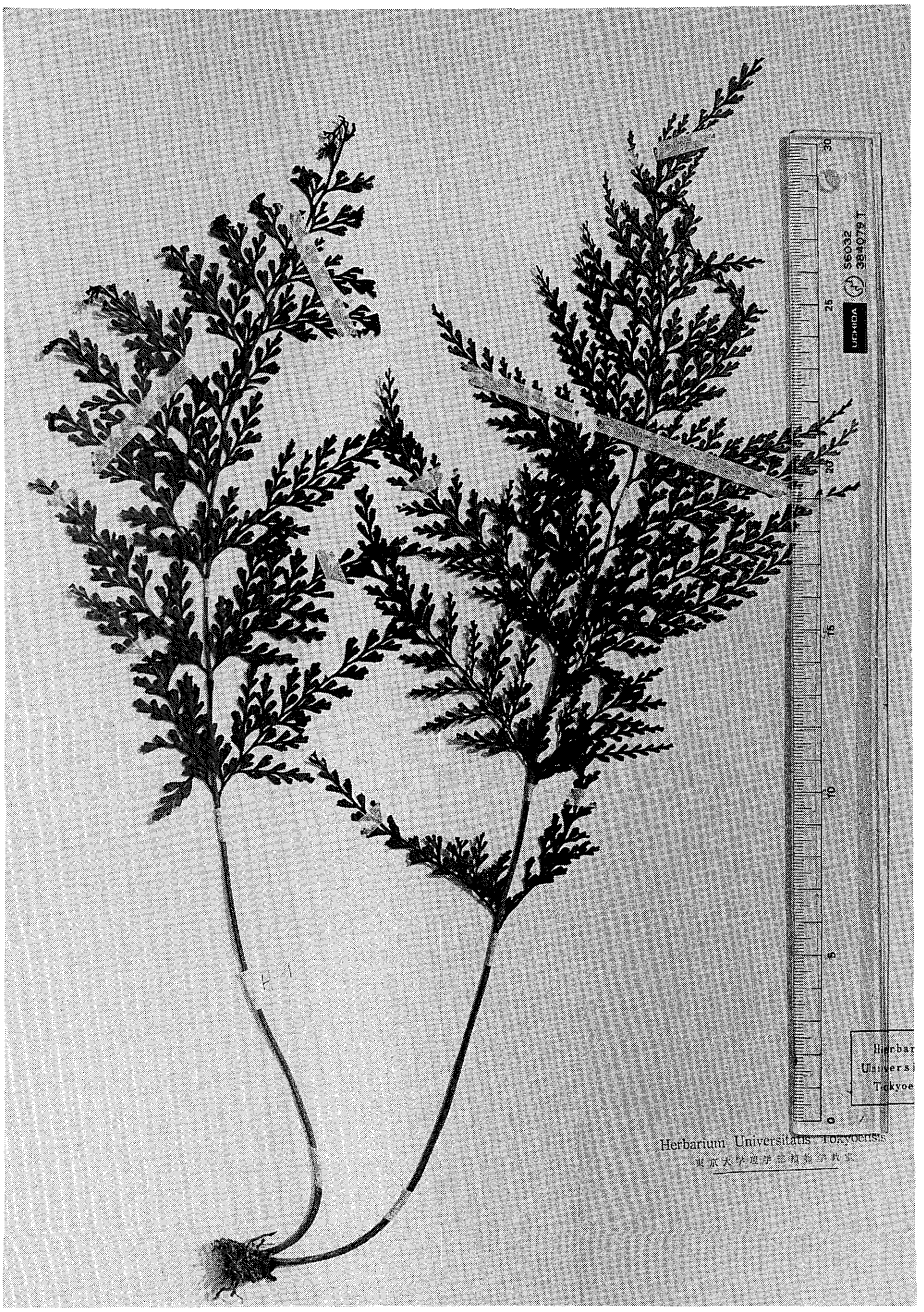

Fig. 6. *Sphenomeris intermedia*. Holotype (Lin 93078).

rowed at base; another form triangular-lanceolate, 4(5) cells at base, gradually acuminate upwards; the last form acicular, consisting of the triangular basal portion and the 1-cell-rowed distal portion. Leaves 20–50 cm long; stipes reddish or at least dark-brown when fresh and dark-brown when dried, as long as lamina or rarely shorter; laminae subcoriaceous, shiny, oblong-lanceolate, with the basal pinnae always shorter than the second basal; pinnae triangular-lanceolate, sometimes with caudate apex, bipinnate + pinnatifid; lobes of sterile leaves oblong-lanceolate, dentate, those of fertile leaves broad-cuneate or cuneate, 1.5–3 mm wide, 3–4 mm long; bicellular glandular hairs c.160 μm long, sparse or dense. Sori each on 1(2) vein-ending; indusia (1)2 cells thick, rigid, convex at the base, either adnate along the entire sides, round at the free apical margin, and opaque, or adnate only 4/5 at the sides, dentate at the apical margin, and subtransparent. Spores 32 per sporangium [but sexual in reproduction, as in other species of *Sphenomeris* (Lin et al. 1990)], monolete, ellipsoid, smooth.

Type. Japan. Okinawa Pref. (Ryukyu Islands): Ishigaki Island, Mt. Banna: on an open sunny slope of a hill, Lin 93078 (TI).

Specimens examined. Okinawa Pref.: Iriomote Island, along a road on a slope between Ohtomi and Komi, Lin 93056; Ishigaki Island, Mt. Omoto, along a path on a slope in Mt. Omoto, Lin 93086, 93098, 93099; Mt. Banna, Lin 93062, 93064, 93065, 93066, 93067, 93068, 93069, 93073, 93074, 93076, 93079, 93097.

Japanese name. Ainoko-horashinobu.

Distribution. Southern Ryukyu Islands (Iriomote Is. and Ishigaki Is.).

Ecology. In open places usually along roads or paths inland some distances from the coast.

Note. This species is intermediate between *S. chinensis* and *S. biflora*, but can be distinguished from *S. chinensis* by the narrowly triangular scales with the 4–5 cell-rowed base, shiny laminae, and 2-cells thick indusia. It differs also from *S. biflora* in having lamina oblong-lanceolate, basal pinnae shorter than the upper, and stipes reddish when fresh and dark-brown when dried.

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Appendix: Herbarium specimens examined*

Sphenomeris biflora

Okinawa Pref.: Iriomote Isl. near Sonai, 16–18 Feb. 1958, R. Nozu s.n. (TI); Komi, Iriomote Isl., 29 March 1963, R. Manyo s.n. (TOFO).

Bonin Isls.: Chichijima Isl. Yankeetown, 13–14 Feb. 1950, F. R. Fosberg 31504 (TI).

Kagoshima Pref.: Amami Isl. et Edateku Isl., 2–7 March 1966, S. Sako 6122 (TI); Amami Isl., Sumiyo-mura, 20 Aug. 1959, K. Iwatsuki 5046 (TI); Amami Isl., Kasari-mura, 17 Sep. 1956, S. Sako 150 (TI); Amami Isl., 8 Aug. 1959, M. Hutoh 21395 (TOFO); Amami Isls., Tokunoshima, Kimotsuki-gun, Sata-cho, 6 July, 1974, T. Yamazaki et al. 444 (TI); Yakushima Isl., near Nagata, 21 Aug. 1957, K. Iwatsuki 3339 (TI); Yakushima Isl., Kamiyaku-mura, 18 Nov. 1957, M. Hutoh 18662 (TOFO); Shimokoshiki Isl., Teuchizaki, 8 Jan. 1960, M. Hutoh 21941 (TOFO); Shimokoshikishima Isl., 24 April 1970, M. Takashi s.n. (TI); Nagasakibana, Ibusuki-gun, H. Ohba & S. Akiyama 251 (TI); Akuseki, Isls. Takara, Ohsumi, 25–30 March 1966, S. Sako 6139 (TI).

Kumamoto Pref.: Amakusa, Shimoda, western Peninsula, 26 Dec. 1968, M. Kido 10045 (TOFO).

Mie Pref.: Kuki-zaki, near Owase, 16 Nov. 1958, Y. Higuchi s.n. (TI).

Wakayama Pref.: Taichi-cho, Higashimuro-gun, 17 Oct. 1960, H. Hiroe 14816 (TI); Taichi, Higashimuro, 23 Nov. 1970, S. Mitsuta s.n. (TOFO).

Shizuoka Pref.: Izu Peninsula, Atagawa, 18 May 1958, K. Iida s.n. (TOFO); Inatori, 23 June 1957, K. Satake s.n. (TOFO); Sotowura, Shimoda, 14 Nov. 1956, K. Satake s.n. (TOFO). Izu Isls. (Tokyo Metro.): Archipelago, 22 Nov. 1964, H. Ohba 878 (TI); Aogashima, 11 Nov. 1954, M. Mizushima s.n. (TI); Hachijojima, 18 July 1933, T. Tuyama s.n. (TI); Kouzu Isl., 22 Dec. 1965, H. Ohba 2076, 2024 (TI); Mikurajima Isl., July–Aug. 1967, S. Masuda et al. s.n. (TI); Miyakejima, 12 Oct. 1963, N. Satomi 22345 (TI); Ohshima Isl., 18 March 1971, Y. Tateishi 238 (TI); Ohshima Natural Park, 8 Oct. 1967, F. Kurihara et al. 3607 (TI); Natural Zoo of Izu-Ohshima, 22 Nov. 1964, H. Ohba 869 (TOFO); Miyakejima, 19 Nov. 1951, S. Kurata 1351 (TOFO).

Kanagawa Pref.: Yokohama, Hommoku, 3 May 1962, Saito s.n. (TOFO).

Ibaraki Pref.: Hitachi, Hamanomiya Peninsula, 22 Nov. 1972, M. Yasu s.n. (TOFO).

Taiwan: Lanyu, 4 Aug. 1968, T. Nanba et al. s.n. (TI).

Hong Kong: Green Isl., 28 March 1972, S. Y. Hu 11696 (TI).

S. gracilis

Okinawa Pref.: Iriomote Isl., Ohtomi, 6 Feb. 1980, Y. Tateishi et J. Murata 4749, 4725 (TI); Iriomote Isl., Nakama River, 3 July 1881, Y. Miyagi 9507 (TI); Iriomote Isl., Shirahama, 17 May 1936, Y. Ito s.n. (TI); Hinai River, Funaura, 8 March 1982, M. Kato et al. 510 (TI); Iriomote Isl., July 1923, G. Koidzumi 349-105 (isotype) (KYO); Iriomote Isl., Hoshitate, 11 Aug. 1980, K. Shimabuku 5153 (349-102) (KYO); Ishigaki Isl., 29 July 1964, anonym. coll. (TOFO).

S. chinensis

Okinawa Pref.: Iriomote Isl., Urauchi River, 29 Dec. 1972, Saito s.n. (TOFO); Iriomote Isl., Sonai–Shirahama, 1 Aug. 1959, K. Oka 13676 (TOFO); Iriomote Isl., Kanpira waterfall, 25 March 1968, T. Nakaike 2269 (TOFO); Iriomote Isl., Tomihara, 26 March 1963, Biological Dept., Univ. of Waseda s.n. (TOFO); Iriomote Isl., Shirahama, 20 March 1973, H. Koyama et al. 799 (TI); Kohamajima Isl., 7 July 1975, K. Shimabuku 3114 (TI); Okinawa Isl., Mt. Yonaha, 13 Oct. 1973, S. Kurata s.n. (TOFO).

Bonin Isls.: Chichijima Isl., 6 July, 1975, G. Murata et al. 189 (TI).

Kagoshima Pref.: Ibusuki, 1 Aug. 1977, K. Kawahara 1555 (TOFO); Satsuma, Shimokoshiki Isl., near Teuchi, 27 Oct. 1968, M. Furuse 129 (TOFO); ibid. 9 Jan. 1960, M. Hutoh 21960 (TOFO); Yakushima Isl., Hanaage River, 20 Aug. 1973, R. Oka s.n. (TOFO); Yakushima Isl., Kosugi-dani, 19 Dec. 1961, S. Kurata 5611 (TOFO); Amami Isls., Kikai Isl., 15 Aug. 1959, M. Hutoh 21628 (TOFO); Kimotsuki-gun, Sata-cho, Odomari–Sotomura, 6 July 1974, T. Yamazaki et al. 415 (TI); Kagoshima, Hirakawa–Eboshidake, 8 July 1974, T. Yamazaki & H. Ohba 514 (TI); Ibusuki-gun, Kaimon-cho, Mt. Kaimondake, July, H. Ohba & S. Akiyama s.n. (TI).

Nagasaki Pref.: Mt. Kazusa-Iwato, 23 July 1961, H. Arao 140 (TOFO); Tsushima Isl., 1 Aug. 1967; N. Miake s.n. (TOFO); Tsushima Isl., Omoda, 7 May 1962, M. Ohno s.n. (TOFO); Tsushima Isl. Shimoagata, 1973, H. Ohashi & H. Ohba 307 (TI); Nagasaki City, Uehara-cho, 6 Jan. 1977, B. Matsubayashi s.n. (TOFO); Hirado Isl., Mt. Yasuman-dake, 23 Aug. 1968, B. Matsubayashi 38 (TOFO).

Kumamoto Pref.: Ushifuka, Mt. Gongen, Aug. 1967, Y. Kobayashi 72 (TOFO); Miyano–Kawachi, 1965, Y. Kobayashi 80 (TOFO); ibid. 18 Oct. 1927, K. Maebara 2550 (TOFO); ibid. 7 June 1939, K. Maebara 3638 (TOFO); Amakusa, Kamishima, 8 May 1946, K. Yamazaki s.n. (TOFO).

Oita Pref.: Amabe-gun, Meiji-mura, 23 Sep. 1960, M. Hadano K67 (TOFO).

Kochi Pref.: Ryu, Usa-cho, S. W. of Kochi, 24 Sep. 1957, M. Tagawa & K. Iwatsuki 2067 (TOFO).

Ehime Pref.: Omishima Isl., Miyaura, 16 July 1959, K. Ochi 15272 (TOFO); Sata Cape, Misaki, 26 July 1961, Y. Miyoshi s.n. (TOFO).

Yamaguchi Pref.: Kumage-gun, Marifu-mura, 22–23 July 1935, F. Maekawa s.n. (TI).

Kyoto Pref.: Kyoto, Shishigatani, 3 Oct. 1955, M. Tagawa 7149 (TI).

Mie Pref.: Owase, Kuki, Namera-dani, 27 July 1981, H. Ohba & S. Akiyama 514 (TI).

Aichi Pref.: Nishio City, Yaojite, 4 March 1978, Y. Inami

s.n. (TOFO).

Shizuoka Pref.: Kakegawa, Ketsuenzi Itasawa, 3 Feb. 1978, H. Ohasi et al. 1357 (TI).

Izu Isls. (Tokyo Metro.): Miyake Isl., Kamitsuki, 31 Nov. 1933, K. Hayashi s.n. (TOFO); Miyake Isl., Tsubota, 20 Nov. 1951, S. Kurata 1366 (TOFO).

Kanagawa Pref.: Fujisawa, Mutsuaimura, Oct. 1953, Y. Asai s.n. (TI).

Chiba Pref.: Mt. Kiyosumi, 23 Oct. 1949, M. Nishida 655 (TI).

Ibaraki Pref.: Hitachi, Takehagi-cho, 21 July 1948, S. Kurata 545 (TOFO).

Niigata Pref.: Kakizaki, Hirasawa, Mt. Yoneyama, 16 Nov. 1965, E. Sasama 2 (TOFO).

S. minutula

Kagoshima Pref.: Amami Isl., Sumiyo River, 30 Oct. 1969, Y. Inoue s.n. (TOFO).

*Species of *S. intermedia* sp. nov. are cited below the description of the species.

林　蘇娟，加藤雅啓，岩槻邦男：**日本産ホラシノブ属（ホングウシダ科）の分類学的研究**

日本にホラシノブ属は2種分布するとみるのと，4種が存在するというのと見解が分かれている．日本産の種をはっきりと認識するために形態，生態および細胞学的観察をおこなった．その結果，ホラシノブ，ハマホラシノブ，ヒメハラシノブ（八重山群島固有），コビトホラシノブ（奄美大島固有）の4種が形態形質，生育環境，染色体基本数から区別できた．ホラシノブの2倍体と4倍体のサイトタイプも形態的に（孔辺細胞，胞子，腺毛で）識別できた．ホラシノブ2倍体とハマホラシノブの雑種，ホラシノブとヒメホラシノブの雑種は減数分裂と胞子形成が異常であったので，それらの種は互いに生殖的に隔離されていると思われる。ホラシノブ4倍体とハマホラシノブの雑種も存在する．上の4種のほかにホラシノブとハマホラシノブの間の雑種起源の異質4倍体種と考えられる新種 ***Sphenomeris intermedia***（アイノコホラシノブ）が八重山群島に分布することが明かになった．